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Lymphadenopathy Associated Virus and Its Etiological Role in AIDS

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Abstract: Lymphadenopathy associated virus (LAV) is a novel human retrovirus first reported in 1983. It was isolated from the lymph node lymphocytes of a French homosexual patient with generalized hyperplasic lymphadenopathy. Subsequently LAV was isolated from patients with frank acquired immune deficiency syndrome (AIDS) coming from all the different high-risk groups, while anti-LAV antibodies were detected equally in individuals from all "at-risk" groups. Such a profile is consistent with the virus being the major etiological agent of AIDS. Furthermore its biological properties, namely its cytopathic effect *in vitro*, its T4-cell tropism as well as the role of the T4 molecule in virus infection explain, at least in part, the pathophysiology of AIDS. The major core (*gag*) proteins are p18, p25, and p13 which are products of a Pr55 precursor. The major envelope (*env*) glycoprotein is unusually large (gp110) for a retrovirus and comparable to those of the lentiviruses. Recently the virus has been molecularly cloned. The genome is 9.2 kb long, longer than any other known replication competent retrovirus apart from the lentiviruses. The absence of molecular hybridization between cloned LAV and human T-cell leukemia/lymphoma virus (HTLV) genomes compounds the original and extensive differences noted between these viruses and demonstrates that LAV is a prototype of a new class of human retrovirus.

Historical Background

The discovery of the etiological agent of acquired immune deficiency syndrome (AIDS) exemplifies the ebb and flow of ideas and interpretations, frustrations, and false starts, data and dead ends which often characterize scientific progress until one clear and overwhelming hypothesis emerges. That human T-cell leukemia/lymphoma virus type I (HTLV-I), or a virus closely related to it, was the causative agent of AIDS was a hypothesis developed in 1983 by Essex and Gallo (*1, 2*). Already Essex and coworkers had demonstrated that feline leukemia virus, a lymphotropic retrovirus, was capable of not only inducing leukemia but could also induce chronic and profound immune depression. It was therefore plausible at the time to think that

HTLV, then the only known human retrovirus, or a virus closely related to it, was likely to be the AIDS agent. Indeed, some of the earlier data published by the two groups seemed to support this hypothesis: HTLV-I hybridizing sequences were found in lymphocyte DNA of two AIDS patients (*2*) out of 33, and HTLV-I was isolated from three AIDS patients (*3*). A large number of sera of AIDS patients reacted in a membrane fluorescence assay (HTLV-MA) with an antigen, assumed to be related to HTLV-I envelope protein, present at the surface of an HTLV-I transformed cell line (*1*).

At the same time we, at the Pasteur Institute, were characterizing a novel retrovirus that we had isolated by culturing lymphocytes derived from a biopsied lymph node of a patient with persistent generalized lymphadenopathy. This virus clearly differed from HTLV-I but it was thought at first to belong to the same group (*4*). The major core protein of this virus, p25, was not antigenically related to that of HTLV-I, but serum of the patient reacted by immunofluorescence with fixed cells of two HTLV-I transformed cell lines, suggesting that a viral protein had a common antigenic site with that of HTLV-I. In the following 3 months new data strongly suggested that, in fact, we were dealing with a new retrovirus, not at all related to the HTLV group, and one which looked increasingly like the best candidate agent of AIDS. Such evidence stemmed from many observations, namely, i) the peculiar morphology of the virus, ii) its isolation from all groups of patients with AIDS, related syndromes or at risk for AIDS, iii) its cytopathic effect on T4 lymphocytes *in vitro*, and iv) the proportion of pre-AIDS or AIDS patients positive for lymphadenopathy associated virus (LAV) antibodies against viral proteins was larger than that positive for HTLV-I antibodies. These data were presented at a night session of the HTLV meeting at Cold Spring Harbor on September 15, 1983 (*5*). A new name was given to the isolates, LAV or immune deficiency associated virus (IDAV). However, the majority of the audience was rather skeptical, the dominant concept at the time still being that an HTLV-I variant was the cause of AIDS. However, the body of evidence for the HTLV-I like agent was rapidly shrinking. It was clear that the geographical distribution of AIDS did not coincide with that of HTLV endemic areas; in the southwest of Japan, AIDS was non-existent. Conversely, AIDS patients were not infected with HTLV-I in Western Europe where its prevalence is extremely low. The situation in the U.S.A. and Caribbean was less clear which helped maintain the confusion. Some patients were clearly doubly infected with HTLV-I and the new LAV HTLV-I being either an additional cofactor or another opportunistic agent. The specificity of the HTLV-MA test was still an open question. At the UCLA meeting organized in Park City in February 1984, the tide had turned. Most of the attendants realized, after the presentation of our work, that LAV and not HTLV-I or -II was the right candidate (*6*). Following two more publications (*19*, *32*), several publications by Gallo's group described in May 1984 under the name of HTLV-III a group of viruses which they were able to grow in large amounts in a continuous T-cell line, and which had the same properties as LAV (*7*, *10*).

It is now certain, after the viruses have been exchanged, that they are closely related to each other as well as to the isolates made at the CDC (*11*), at San Francisco University (*12*), and in other laboratories. All these isolates can be considered as different isolates of the LAV prototype. However, a matter of debate remains: to

what degree does LAV resemble an HTLV virus? We now know that this virus is very different from the HTLVs (it cannot even be considered a cousin) and, if anything, shows some features in common with the lentiviruses (*vide infra*).

Physico-chemical and Antigenic Properties of the Virus

1. Proteins

1) Core

The major protein of the viral core is a methionine rich protein, with apparent molecular weight of 25,000 daltons (p25) in SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Most, but not all pre-AIDS and AIDS patients, as well as healthy carriers, have antibodies against p25 (*13–15*). When the virus is mildly treated with a non-ionic detergent, the p25 is associated with particles banding at 1.24 in sucrose density gradient, presumably viral cores. Two other proteins, p18 and p13, presumably also associated with the internal part of the virion, are not well labelled with ^{35}S-methionine and are presumably methionine poor. These proteins are apparent on gels after silver staining or after labelling with ^{14}C-amino acids or ^{35}S-cysteine. Some of the patients sera have antibodies against p18 and p13. A few have antibodies against p18, while lacking anti p25 antibodies. The significance of these differences remains to be determined.

2) Envelope glycoprotein

Until very recently, there was some uncertainty about the viral envelope proteins. We have recently succeeded in identifying the major glycoprotein of 110,000 daltons (gp110), which is one of the largest known amongst retroviruses. This was achieved by heavily labelling the virus by ^{35}S-cysteine followed by immunoprecipitation using patients' sera. It should noted that nearly all patients have antibodies against LAV gp110, without detectable or neutralizing action on viral infectivity *in vitro*. Only two infants with AIDS (5 and 9 months old, respectively) lacked antibody against this protein, as well as against other viral proteins. Presumably they were infected at birth by their mother and were unable to mount an antibody response. The glycoprotein nature of this protein was demonstrated by the reduction of its apparent molecular weight after treatment by an endoglycosidase and its affinity binding to columns of Concanavalin A (ConA) or lentil-agarose. The incorporation of the gp110 in virions was shown by its association with the band of purified virus in a Nycodenz gradient. Mild treatment with non-ionic detergent releases it from the viral particle.

The relationship of this protein to the p41 detected by the Western blot technique by Gallo and coworkers is at present unclear. It may represent another processing of the *env* gene product or, alternatively, a cellular protein specifically induced by viral infection in T4 lymphocytes. It is likely that knowledge of the viral genome structure will help to clarify this point. Other minor proteins, present in immunoprecipitates, are under study.

2. Viral genome

The complete LAV genome has been cloned. The cloning strategy followed was firstly the cloning of endogenously synthesized cDNAdT into pBR327. A family of cross hybridizing recombinants, of which pLAV13 carried the longest insert (2.5 kb),

was isolated. This LAV specific probe was then used to screen a genomic library of DNA made from LAV infected cells. In this way genomic clones representing the complete integrated proviral DNA were isolated. It must be stressed that while the cDNA was made from the continuously LAV producing B-cell line, FR8, the complete genomic clones were isolated from the DNA of phytohemagglutinin (PHA)-activated lymphocytes of a normal donor (C6) which had been infected for 5 days with a stock of LAV1 that had never been previously passed in continuous B- or T-cell lines. In this way we eliminated the possibility of cloning either an attenuated virus which may be present in permanent tumor cell lines or a virus subtlely different in the long terminal repeat (LTR), as has already been noted for leukemic and non-leukemic strains of a murine retrovirus.

Two molecular clones of 9.1 kb long were isolated differing in one *Hin*dIII restriction site. This viral heterogeneity was also evident in the Southern blot of *Hin*dIII restricted DNA. Thus the virus is heterogeneous even within a single isolate and it is to be anticipated that between LAV isolates considerable differences will be observed.

Comparison with the HTLVs and Other Retroviruses

From the moment that LAV was first described it became a subject of intense interest as to whether or not the virus belonged to the HTLV family of retroviruses (*18*). Morphologically the virus was very different but only the biochemical characterization to the viral protein and nucleic acid, and ultimately the nucleotide sequence, could really nail down this point. The major core protein migrates slightly more slowly in SDS-PAGE than p24s of HTLV-I and -II. No antigenic cross-reactivity could be found by radioimmuno-competition between LAV p25 and HTLVs p24 (*4*), nor with the core proteins of murine, bovine or primate retroviruses. The p26 of equine infectious anemia virus (EIAV) and the p25 of LAV share a common epitope, since the serum of some EIAV infected horses immunoprecipitates LAV p25 (*19*), but antibodies against this epitope do not seem to exist in sera of human patients infected with LAV. The very high-molecular weight glycoprotein is a unique feature of LAV (or HTLV-III) among human retroviruses.

The glycoproteins of HTLV-I and -II have molecular weights of 46, 61, and 68 kdaltons (*20*, *21*). Antibodies against a synthetic peptide of the gp of HTLV-I (courtesy Dr. M. Yoshida) did not immunoprecipitate LAV gp110. Only the lentiviruses (visna maedi, caprine arthritis encephalitis virus) have such a high-molecular weight glycoprotein (*22*).

The restriction map of cloned LAV does not bear any resemblance to those of HTLV-I, HTLV-II or even bovine leukemia virus (BLV) which is a distant relative of the HTLVs (*16*). Attempts to hybridize cloned LAV and HTLV-II even under extremely relaxed conditions (Tm−55°C) came to nothing, a result in sharp contrast to those of Gallo and coworkers who reported hybridization between the *gag-pol* and X regions of HTLV-I to cDNA and molecularly cloned HTLV-III under moderately stringent conditions (Tm−28°C) (*23*, *24*). This discrepancy is particularly surprising as the cloned LAV and HTLV-III genomes differ only by a single restriction site, and then this "absent" restriction site is found in a cDNA clone of LAV.

Subsequently it was reported that in fact there was very minimal, if any, hybridization between the X region of cloned HTLV-I and HTLV-III (*25*). It seems that only a direct comparison of the nucleotide sequences of LAV (or HTLV-III) and HTLV-I will resolve this discrepancy. Indeed, the LAV/HTLV-I comparison reveals no sequence homology whatsoever in the X region and only a 16 bp tract in the *gag-pol* region of HTLV-I (S. Wain Hobson and M. Alizon, unpublished results).

The comparison of the LAV glycoprotein with those of the lentiviruses is the more interesting since the only retroviral genomes of comparable length to that of LAV (9.1 kb) are those of the visna and CAEV lentiviruses (*26, 27*). However, we could not find any DNA hybridization between cloned visna and LAV genomes under extremely low stringency (Tm−55°C) (*16*).

Biological Properties

1. Requirement for lymphocyte activation

Fresh T lymphocytes do not spontaneously express whole virus. Previous activation by lectins (PHA, ConA, Pokeweed mitogen, wheat germ agglutinin), alloantigens or bacterial toxins is required for virus expression. The presence of antibodies against α interferon also greatly enhances propagation of the virus into lymphocytes of healthy donors, suggesting a strong control of virus production of endogenous α interferon (*5*). Virus production by normal lymphocyte cultures was only transient and was in all cases followed by a decline of cell multiplication. Immortalization of T or B cells has never been observed, unlike what is commonly seen with HTLVs.

2. Tropism for lymphocytes expressing the T4 surface marker

This tropism was demonstrated *in vivo* and *in vitro* by experiments described in detail elsewhere (*5, 28*). After separating T lymphocytes into T4 and T8 subsets by means of cellular chromatography with specific monoclonal antibodies, only T4 lymphocytes were found able to express the virus or to be infected by the virus. Recently, we have been able to show that the T4 molecule itself may serve as receptor for the virus. Viral infection of normal lymphocytes was inhibited in the presence of monoclonal antibodies against the T4 molecule (*29*). Using vesicular stomatitis virus (VSV) pseudotypes, Weiss and colleagues have obtained similar results (*30*). These results do not exclude other surface proteins being involved in adsorption and penetration of the virus. Despite such strong tropism, lymphocytes harboring the B phenotype can also be infected under the same conditions.

Using the LAV1 strain (isolated from a lymphadenopathy patient), it was possible to infect a lymphoblastoid line obtained after *in vitro* transformation of B lymphocytes with Epstein-Barr virus (EBV). In the original experiment, the B lymphocytes came from the same donor (FR) as the T lymphocytes used to grow the virus. Later on, it was found that the virus could also grow, after a one month lag, on some but not all lymphoblastoid cell lines derived from other individuals. The virus propagated in the FR8 lymphoblastoid cells underwent some adaptation to B cells, since it grew without latency on these B-cell lines and also could grow on a BJAB line derived from an African Burkitt lymphoma. This line is of particular interest, since it is free of the EBV genome and can be supertransformed *in vitro* by

EBV. Infection of an EBV negative cell line by LAV1 indicates that EBV is not necessary for the retrovirus infection. However, upon transformation of the cell line by EBV, the yield of the retrovirus appeared to be greatly enhanced, suggesting some positive interaction between LAV and EBV (*22*). The molecular basis for infection of B cells with LAV is not known. In principle, such cells do not harbor the T4 marker, although recent data (*21*) suggest that this could be the case in a lymphoblastoid cell line permissive for LAV infection.

The possibility that EBV transformed lymphoblastoid cells may serve as a reservoir for LAV replication in the immunodepressed host cannot be discarded. However, it should be stressed that only the LAV1 strain has demonstrated so far such a broad tropism and that three other LAV strains freshly isolated from AIDS patients were not able to grow in lymphoblastoid cell lines. Apparently, the LAV1 strain had undergone some adaptation due to its long propagation *in vitro*. Recent data obtained in our laboratory suggest that passaging on bone marrow cells is critical in such adaptation.

The biochemical and antigenic properties of the virus "B" LAV remain unchanged, as well as its selective tropism for the T4 subset of normal T lymphocytes. It will be interesting to study its nucleotide sequence, in order to understand the molecular basis for its change in tropism.

3. Induction of cytopathic effect in target cells

Unlike HTLV-I and -II, LAV has never been associated with lymphocyte transformation. This was already clear from the first isolation of the virus. The premature death of infected lymphocyte cultures suggested that the virus could instead induce a cytopathic effect. However, a clear cytopathic effect could be seen only when the titer of the virus propagated on normal lymphocytes was high enough to infect simultaneously a sufficient number of cells. Giant cells with peripheric localization of nuclei arising by cell fusion could then be seen. Some of these giant cells were releasing a number of viral particles. As for other retroviruses, this effect is probably due to the viral glycoprotein. Death of the giant cells occurred within 24 hr after formation. Besides this extreme effect, a general inhibition of the multiplication of T4 lymphocytes was also observed. Curiously, a disappearance of T4 and to a less extent of T3-cell surface markers occurred at the time of virus production (*28*). This phenomenon can best be explained by the binding of viral particles or free viral glycoprotein on these antigens.

Involvement of LAV in AIDS Pathogeny

The above described properties of LAV fit well with what should be expected for the AIDS agent: tropism for the $T4^+$ lymphocytes which are precisely the main cellular subset affected in the disease. This results in either a cytopathic effect as observed *in vitro* or a possible impairment of their function. In the absence of an animal system in which the virus can reproduce the disease, two other requirements should be met: 1) isolation of the virus from AIDS and pre-AIDS patients belonging to all groups, 2) prevalence of antibodies to the virus in the patients and in the various groups of population at risk.

TABLE 1.

A: LAV isolates from French patients

No.	Patient's initials	Disease	Group	Serology LAV
1	RUB	LAS	Homosexual Caucasian	+
2	LAI	LAS AIDS (KS)	Homosexual Caucasian	+
3	DL	AIDS (Tx)	Hemophiliac B Caucasian	+
4	EL	None	Hemophiliac B brother of patient 3	+
5	CHA	LAS AIDS (KS)	Homosexual Caucasian Stay in Haiti in 1980–1981	+
6	ALL	LAS	Caucasian living in Trinidad	+
7	REM	LAS AIDS (PC)	Homosexual Caucasian	+
8	MCC	LAS	Homosexual Caucasian	+
9	STR	AIDS	Bone marrow transplant Caucasian	+
10	BED	AIDS	Bone marrow transplant Caucasian	+
11	CHAR	LAS	Homosexual Caucasian	+
12	LEM	LAS	Homosexual Caucasian	+

B: LAV isolates from African and Haitian patients

No.	Patient's initials	Disease	Group	Serology LAV
13	ELI	AIDS (PCP)	Zairian	+
14	NDO	AIDS (CR)	Zairian	+
15	MUN	ARC wife of patient 14	Zairian	+
16	ED	LAS (Tb)	Haitian	+
17	LUB_M	AIDS mother of patient 19	Haitian	+
18	LUB_F	ARC father of patient 19	Haitian	+
19	LUB_{MIC}	AIDS son	Haitian	+

C: LAV isolates from patients in the U.S.A.

No.	Disease	Group	Serology LAV
20	AIDS (PCP)	IV drug addict NY	+
21	AIDS (PCP)	IV drug addict NY	+
22	AIDS (KS)	Homosexual NY	+
23	AIDS (PCP)	Transfusion blood donor LA	+
24	AIDS (PCP)	Transfusion blood recipient LA	+
25	AIDS (KS)	Homosexual LA	+
26	AIDS (PCP)	IV drug addict NY	+
27	AIDS (PCP)	IV drug addict NY	+
28	AIDS	Homosexual SF	+
29	AIDS	Homosexual NY	+
30	AIDS	Homosexual NY	+
31	LAS	Homosexual (Calif.)	+
32	LAS	Homosexual (Calif.)	+
33	LAS	Sexual contact of patient 31 Homosexual (Calif.)	+
34	AIDS (KS+PCP)	Sexual contact of AIDS patient Homosexual NY	+

IV, intravenous.

1. Frequent isolation of the virus from AIDS and pre AIDS patients

Since isolation of the virus was a time- and person-consuming task, our initial strategy has been to isolate the virus from all patient groups, which include homosexuals, hemophiliacs, Africans, and Haitians.

Soon after LAV1 isolation, we were able to isolate similar viruses from two hemophiliac brothers (one having AIDS, the other being asymptomatic) (*32*) from a Zairian woman who died in France from AIDS (*5*), from a Zairian couple, one with AIDS, one with lymphadenopathy (*33*), and from Haitians (*33*). All these isolates have in common the typical morphology, an antigenically related p25 and the property of transient growth in T lymphocytes with induction of giant cells. The first three isolates from AIDS patients were designated as IDAV. However, the nomenclature has not been widely used, and we prefer to call them LAV followed by a number in chronologic order. The 30 isolates are described in Table 1. It should be noted that LAV has also been detected in cultured lymphocytes from a normal healthy female from Martinique not belonging to any high risk group. The virus could not be propagated in lymphocytes from normal donors and was only proven to be similar to LAV by molecular hybridization under high stringency with a LAV1 DNA clone. Whether this virus has the same pathogenicity as the other LAV isolates remains to be determined.

Other attempts to isolate LAV from healthy blood donors have failed. However, appearance of AIDS and isolation of LAV from patients grafted with the bone marrow of healthy donors which themselves do not belong to high risk groups suggest that the virus is more widely spread throughout the general population in France than was originally thought.

2. Serological studies

Several laboratory tests have been used for the detection of LAV antibodies. A radioimmunoprecipitation assay (RIPA) with LAV1 virus metabolically labelled with ^{35}S-methionine allows detection of anti-p25 antibodies, since this protein is methionine rich. The virus is pelleted at high speed centrifugation and lysed by a detergent containing buffer (*4*). After incubation with sera to be tested, the immunocomplexes are bound to protein A Sepharose beads, washed several times and analyzed by SDS-PAGE. More recently, the same assay has been adapted to ^{35}S-cysteine labelled virus. In this case, antibodies against the four major proteins, p25, p18, p13, and gp110 can be detected. In parallel, for epidemiological studies, an enzyme-linked immunosorbent assay (ELISA) has been set up using gradient purified virus, initially produced by freshly infected T-lymphocyte culture and more recently by permanent B- and T-cell lines.

To take into account unspecific protein binding, each tested serum is incubated in parallel with a lysate of uninfected cells (*13*). Blocking experiments with monoclonal antibodies indicate that the main antigen recognized in the initial ELISA was p18 and not p25. Repeated banding of the virus in sucrose gradients leads to a partial loss of the envelope glycoprotein. Recent improvements in the virus yield and purification have increased the sensitivity of the ELISA close to that of ^{35}S-cysteine RIPA. Another sensitive technique used has been the "Western" blotting

technique. In this case, however, the viral proteins recognized by antibodies are denatured, so that some epitopes may be unmasked and some others destroyed. Here again, antibodies to the viral gp110 are missed, unless special precaution in virus purification and protein transfer on nitrocellulose paper are taken. Recently, indirect immunofluorescence on fixed cells from highly infected lines (CEM, MOLT) has also given good results. The main antigen recognized is the viral glycoprotein present at the surface of the infected cells.

Results obtained with these different techniques can best be summarized as follows:

a) *AIDS patients:* Several studies on patients before and after the onset of AIDS indicate a frequent decrease of the antibody response to LAV antigens at the onset of AIDS and even disappearance of antibodies against the core proteins at a late stage of the disease. However, antibodies against gp110 remain detectable by RIPA ^{35}S-cysteine or Western blot. This phenomenon explains why only 40–50% of AIDS Caucasian patients had positive serology in our earlier ELISA (*13*) or by radioimmunoassay (RIA) against LAV p25 (*14*). In contrast, approximately 90% of a group of AIDS patients diagnosed in Zaire had detectable antibodies against p25 and were also positive by ELISA (*15*). This suggests a stronger antibody response in African patients and also indicates that there is no general correlation between the antibody response and disease evolution.

b) *ARC (Lymphadenopathy) patients:* This group displays in general a stronger antibody response than patients with frank AIDS. Antibody against LAV p25 can be detected in 70% of such patients. Between 75 and 90% are also scored as positive by ELISA. The remaining 10% have only antibody against the gp110.

c) *Healthy individuals belonging to high risk groups:* In a group of French homosexuals, the prevalence of antibodies detected by ELISA was around 20%. In a cohort of San Francisco homosexuals studied by the CDC, the proportion of anti-p25 positive varied from 1% in 1978, 24% in 1981 to nearly 60% in 1984 (*34*). Similar progression has been found in intravenous drug users in the U.S.A. and in France. The situation is not better for hemophiliacs. In a group of French hemophiliacs heavily treated with commercial preparations of factors VIII and IX nearly 60% were positive by ELISA. In another group more lightly treated, only 10% were positive. Clearly, viral transmission occurred *via* the anti-hemophiliac preparations made from LAV infected blood donors.

d) *General heterosexual population:* Only 1 out of 300 French blood donors investigated has been found to be positive (0.3%). However, we have recently observed familial cases of seropositivity, one member of the family having AIDS, though it could be considered as belonging to a high risk group. For instance, an aplastic patient from Brittany was bone marrow grafted in 1979, the donor being his healthy brother. The patient seroconverted soon after the bone marrow graft and had AIDS 5 years later. The brother was also LAV positive, without any immunological or clinical sign of immune depression. He had no sexual relation at the time he donated bone marrow.

This example, and several others, suggests that LAV infection was sporadic in France before the occurrence of AIDS. In equatorial and subequatorial West

Africa, the spread of the virus through the general population seems to have occurred earlier. In 1980, 5% of a group of young Zairian mothers was already LAV seropositive by ELISA (*15*).

Thus, both the requirements of the isolation of LAV from AIDS and pre-AIDS patients as well as high seropositivity in the groups at risk have been amply met.

CONCLUSION

It is clear from all of the above data that the LAV retrovirus is indeed the etiologic agent of AIDS and related syndromes in humans.

1. Up to now, isolates have been almost exclusively obtained from AIDS and LAS patients, contacts and at-risk individuals (*5*).
2. Seroepidemiological surveys show that LAV seropositivity is significantly observed only among individuals of the same groups.
3. LAV specific tropism for the T4+ lymphocyte subset through its privileged interaction with the very T4 molecule (*29*) and its *in vitro* biological properties—inhibition of lymphocyte proliferation and cytopathic effect (?)—are exactly those that would be expected from an AIDS etiologic agent.
4. Since the first isolation of LAV, other isolates of the same virus, sometimes called HTLV-III (*7*) or ARV (*12*) but with the same properties as LAV, have been obtained under similar conditions throughout the world.
5. Short of the so-called Koch postulate, the best "experimental" evidence of causal relationship between LAV and AIDS comes from documented cases of accidental transmission of the virus and the disease by blood transfusions.

It is nevertheless obvious that LAV may also cause inapparent infection and remain latent at least for years in some individuals. The "cofactors" which contribute to the subsequent development of LAS or AIDS are presently unknown. One perhaps may activate lymphocytes in which LAV DNA is integrated and thus trigger virus expression and spreading to other activated uninfected lymphocytes. It is also possible that other cells than mature T4+ lymphocytes may serve as a reservoir for the virus.

The answer to all these questions will ultimately come from cooperative research programs associating clinicians, virologists, immunologists, and molecular biologists.

REFERENCES

1. Essex, M., McLane, M. F., Lee, T. H., Falk, L., Howe, C.W.S., Mullins, J. I., Cabradilla, C., and Francis, D. P. Antibodies to cell membrane antigens associated with human T-cell leukemia virus in patients with AIDS. Science, *220*: 859–862, 1983.
2. Gelmann, E. P., Popovic, M., Blayney, D., Masure, H., Sidhu, G., Stahl, R. E., and Gallo, R. C. Proviral DNA of a retrovirus, human T-cell leukemia virus, in two patients with AIDS. Science, *220*: 862–865, 1983.
3. Gallo, R. C., Sarin, P. S., Gelmann, E. P., Robert-Guroff, M., Richardson, E., Kalyanaraman, U. S., Mann, D., Sidhu, G. D., Stahl, R. E., Zolla-Pazner, S., Leibowitch, V. S., and Popovic, M. Isolation of human T-cell leukemia virus in acquired immune deficiency syndrome (AIDS). Science, *220*: 865–867, 1983.
4. Barré-Sinoussi, F., Chermann, J. C., Rey, F., Nugeyre, M. T., Chamaret, S., Gruest,

J., Dauguet, C., Axler-Blin, C., Vézinet-Brun, F., Rouzioux, C., Rozenbaum, W., and Montagnier, L. Isolation of a T-lymphotropic retrovirus from a patient at risk of acquired immune deficiency syndrome (AIDS). Science *220*: 868–870, 1983.

5. Montagnier, L., Chermann, J. C., Barré-Sinoussi, F., Chamaret, S., Gruest, J., Nugeyre, M. T., Rey, F., Dauguet, C., Axler-Blin, C. *et al.* A new human T-lymphotropic retrovirus: Characterization and possible role in lymphadenopathy and acquired immune deficiency syndromes. Cold Spring Harbor Meeting on "Human T-cell leukemia viruses," *1*: 363–379, 1984.
6. Chermann, J. C., Barré-Sinoussi, F., and Montagnier, L. Characterization and possible role in AIDS of a new human T-lymphotropic retrovirus. *In;* M. S. Gottlieb and J. E. Groopman (eds.), Acquired Immune Deficiency Syndrome, UCLA Symposia on Molecular and Cellular Biology, New Series, vol. 16, pp. 31–46, Alan R. Liss, Inc., New York, 1984.
7. Popovic, M., Sarngadharan, M. C., Read, E., and Gallo, R. C. Detection, isolation and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. Science, *224*: 497–500, 1984.
8. Gallo, R. C., Saluhuddin, S. Z., Popovic, M., Shearer, G. M., Kaplan, M., Haynes, P., Palker, T. J., Redfield, R., Oleske, J., Safai, B., White, G., Foster, P., and Markham, P. D. Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. Science, *224*: 500–503, 1984.
9. Schüpbach, J., Popovic, M., Gilden, R. V., Gonda, M. A., Sarngadharan, M. G., and Gallo, R. C. Serological analysis of a subgroup of human T-lymphotropic retroviruses (HTLV-III) associated with AIDS. Science, *224*: 503–505, 1984.
10. Sarngadharan, M. C., Popovic, M., Bruch, L., Schüpbach, J., and Gallo, R. C. Antibodies reactive with human T-lymphotropic retroviruses (HTLV-III) in the serum of patients with AIDS. Science, *224*: 506–508, 1984.
11. Feorino, M. P., Kalyanaraman, V. S., Haverkos, H. W., Cabradilla, C. D., Warfield, D. T., Jaffe, H. W., Harrison, A. K., Goldringer, D., Gottlieb, M. S., Chermann, J. C., Barré-Sinoussi, F., Spira, T. J., McDougal, J. S., Curran, J. W., Montagnier, L., Murphy, F. A., and Francis D. P. Lymphadenopathy associated virus (LAV) infection of a blood donor-recipient pair with acquired immunodeficiency syndrome. Science, *225*: 69–72, 1984.
12. Levy, J. A., Hoffman, A. D., Kramer, S. M., Lanois, J. A., Shimabukuro, J. M., and Oskiro, L. S. Isolation of lymphocytopathic retroviruses from San Francisco patients with AIDS. Science, *225*: 840–842, 1984.
13. Brun-Vezinet, F., Rouzioux, C., Barré-Sinoussi, F., Klatzmann, D., Saimot, A. G., Rozenbaum, W., Montagnier, L., and Chermann, J. C. Detection of IgG antibodies to lymphodenopathy associated virus (LAV) by Elisa, in patients with acquired immunodeficiency syndrome or lymphadenopathy syndrome. Lancet, *i*: 1253–1256, 1984.
14. Kalyanaraman, V. S., Cabradilla, C. D., Getchell, J. P., Narayanan, R., Braff, E. H., Chermann, J. C., Barré-Sinoussi, F., Montagnier, L., Spria, T. J., Kaplan, J., Fishbein, D., Jaffe, H. W., Curran, J. W., and Francis, D. P. Antibodies to the core protein of lymphadenopathy-associated virus (LAV) in patients with AIDS. Science, *225*: 321–323, 1984.
15. Brun-Vézinet, F., Rouzioux, C., Montagnier, L., Chamaret, S., Gruest, J., Barré-Sinoussi, F., Geroldi, D., Chermann, J. C., McCormick, J., Mitchell, S., Piot, P., Taelman, H., Kapita Bila Mirlangu, Odio, Wobin, Mbendi, N., Mazebo, P., Kayembe Kalambayi, Bridts, C., Desmyter, J., Feinsod, F. M., and Quinn, T. C. Prevalence of antibodies to lymphadenopathy associated retrovirus in African patients with acquired immune deficiency syndrome. Science, *225*: 453–456, 1984.

16. Alizon, M., Sonigo, P., Barré-Sinoussi, F., Chermann, J.C., Tiollais, P., Montagnier, L., and Wain-Hobson, S. Molecular cloning of lymphadenopathy associated virus (LAV). Nature, *312*: 757–760, 1984.
17. Lenz, J., Celander, D., Crowther, L., Patarca, R., Perkins, D. W., and Haseltine, W. A. Determination of the leukaemogenicity of an immune retrovirus by sequence within the long terminal repeat. Nature, *308*: 467–470, 1984.
18. Poiesz, B. J., Ruscetti, F. W., Reitz, M. S., Kalyanaraman, V. S., and Gallo, R. C. Isolation of a new type C retrovirus (HTLV) in primary uncultured cells of a patient with Sézary T-cell leukaemia. Nature, *294*: 268, 1981.
19. Montagnier, L., Dauguet, C., Axler, C., Chamaret, S., Gruest, J., Nugeyre, M. T., Rey, F., Barré-Sinoussi, F., and Chermann, J. C. A new type of retrovirus isolated from patients presenting lymphadenopathy and acquired immune deficiency syndrome: Structural and antigenic relatedness with equine infectious anemia virus. Ann. Virol., *135 E*: 119–134, 1984.
20. Kiyokawa, T., Yoshikura, H., Hattori, S., Secki, M., and Yoshida, M. Envelope proteins of human T-cell leukemia virus: Expression in *Escherichia coli* and its application to studies of *env* gene functions. Proc. Natl. Acad. Sci. U.S., *81*: 6202–6206, 1984.
21. Lee, T. H., Coligan, J. E., Homma, T., McLane, M. F., Tachibana, T., and Essex, M. Human T-cell leukemia virus-associated membrane antigens: Identity of the major antigens recognized after virus infection. Proc. Natl. Acad. Sci. U.S., *81*: 3856–3860, 1984.
22. Clements, J. E., Narayan, O., and Core, L. C. Biochemical characterization of the virus causing leucoencephalitis and arthritis in goats. J. Gen. Virol., *50*: 423–427, 1980.
23. Arya, S. K., Gallo, R. C., Hahn, B. H., Shaw, G. M., Popovic, M., Salahuddin, S. Z., and Wong-Staal, F. Homology of genome of AIDS-associated virus with genomes of human T-cell leukemia lymphoma viruses. Science, *225*: 927–930, 1984.
24. Hahn, B. H., Shaw, G. M., Arya, S. U., Popovic, M., Gallo, R. C., and Wong-Staal, F. Molecular cloning and characterization of the HTLV-III virus associated with AIDS. Nature, *312*: 166–169, 1984.
25. Shaw, G. M., Hahn, B. H., Arya, S. K., Groopman, J. E., Gallo, R. C., and Wong-Staal, F. Molecular characterization of human T-cell leukemia (lymphotropic) virus type III in the acquired immune deficiency syndrome. Science, *226*: 1165–1171, 1984.
26. Harris, J. D., Scott, J. V., Taylor, B., Brahic, M., Stowring, L., Ventura, P., Haase, A. T., and Peluso, R. Visna virus DNA: Discovery of a novel gapped structure. Virology, *113*: 573–583, 1981.
27. Pyper, J. M., Clements, J. E., Molineaux, S. M., and Narayan, O. Genetic variation amongst lentiviruses: Homology between visna virus and caprine arthritis-encephalitis virus is confined to the 5′ *gag-pol* region and a small portion of the *env* gene. J. Virol., *31*: 713–721 ,1984.
28. Klatzmann, D., Barré-Sinoussi, F., Nugeyre, M. T., Dauguet, C., Vilmer, E., Griscelli, C., Brun-Vézinet, F., Rouzioux, C., Gluckmann, J. C., Chermann, J. C., and Montagnier, L. Selective tropism of lymphadenopathy associated virus (LAV) for helper-inducer T-lymphocytes. Science, *225*: 59–63, 1984.
29. Klatzmann, D., Champagne, E., Chamaret, S., Gruest, J., Guetard, D., Hercend, T., Gluckman, J. C., and Montagnier, L. T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV. Nature, *312*: 767–768, 1984.
30. Dalgleish, A. G., Beverley, P.C.L., Clapham, P. R., Crawford, D. H., Greaves, M. F.,

and Weiss, R. A. The CDA (T4) antigen is an essential component of the receptor for the AIDS retrovirus. Nature, *312*: 763–767, 1984.

31. Montagnier, L., Gruest, J., Chamaret, S., Dauguet, C., Axler, C., Guetard, D., Nugeyre, M. T., Barré-Sinoussi, F., Chermann, J. C., Brunet, J. B., Klatzmann, D., and Gluckmann, J. C. Adaptation of the lymphadenopathy associated retrovirus (LAV) to replication in LAV-transformed B lymphoblastoid cell lines. Science, *85*: 63–66, 1984.
32. Vilmer, E., Rouzioux, C., Brun-Vezinet, F., Fischer, A., Chermann, J. C., Barré-Sinoussi, F., Gazengel, C., Dauguet, C., Manigne, P., Griscelli, C., and Montagnier, L. Isolation of a new lymphotropic retrovirus from two siblings with haemophilia B, one with AIDS. Lancet, *i*: 753–757, 1984.
33. Ellrodt, A., Barré-Sinoussi, F., Le Bras, Nugeyre, M.T., Palazzo, L., Rey, F., Brun-Vezinet, F., Rouzioux, C., Segond, P., Caquet, R., Montagnier, L., and Chermann, J. C. Isolation of a new human T-lymphotropic retrovirus (LAV) from Zairian married couple, one with AIDS, the other with the prodromes. Lancet, *i*: 1383–1385, 1984.
34. Centers for Disease Control. Personal communication.

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